Chapter 5

Metabolic control after allogenic rat islet transplantation into a subcutaneously implanted, prevascularized, and replaceable polymeric scaffold

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Abstract

The primary aim of this study was to study the quality of metabolic control of an allogenic rat islet graft transplanted into a subcutaneous placed poly (D,L-lactide-co-ε-caprolactone) (PDLLCL) scaffold in a diabetic rat model. The glucose clearance of this prevascularized and replaceable scaffold was compared with an islet graft transplanted under the kidney capsule.

Islets were isolated from Albino Oxford rats and either transplanted under the kidney capsule or into the PDLLCL scaffold of diabetic nude rats. Scaffolds were subcutaneously implanted four weeks before transplantation to allow vascularization. Normoglycemia (nonfasting blood glucose < 10 mmol/L) was restored within 1 week in both the kidney capsule and the scaffold group. In both groups, normoglycemia was maintained till the end of the study. However, nonfasting blood glucose levels were more tightly regulated in the kidney capsule group as more fluctuations were observed in the scaffold group. The oral glucose tolerance test, which was performed between 8 and 10 weeks after transplantation, showed that glucose was cleared more efficient in recipients of an islet grafts under the kidney capsule as glucose was metabolized with a prompt increase of plasma insulin levels compared to recipients under de skin, but no differences were found in islet survival between both groups. Remarkably, this difference in insulin regulation resulted in more weight gain in the scaffold animals.

Our data demonstrate that a subcutaneous, prevascularized PDLLCL scaffold can obtain metabolic control after allogenic rat islet transplantation in a diabetic rat model. The quality of this metabolic control seemed to be slightly less efficient than after transplantation under the kidney capsule. This difference is probably the consequence of differences in speed and amount of blood supply to islets under the skin and under the kidney capsules. However, the observed efficacy is still acceptable suggesting that our scaffold can be used to create transplantation site for pancreatic islets.

Introduction

Cell replacement is a promising therapy for several diseases, such as Alzheimer [1], Parkinson [2], strokes [3], sickle cell disease [4], hepatic failure [5], and diabetes mellitus [6]. An example of cell replacement is the transplantation of pancreatic islets to restore the glucose homeostasis in type 1 diabetic patients [6,7]. This therapy results in insulin-independence [6,8], but the long-term success rate is still low nowadays [6,9]. To improve long-term survival of islets, an optimal transplantation environment should be created. An optimal transplantation site should provide a vascular network and innervation to ensure adequate nutritional and physical support to the islet graft. Furthermore, it should have an adequate tissue volume capacity, induce a minimal tissue response, and should be easy accessible for transplantation and monitoring of the islet graft.

Recently, we introduced a subcutaneously implanted prevascularized, retrievable poly (D,L-lactide-co-ε-caprolactone) (PDLLCL) scaffold for transplantation of pancreatic islets. This scaffold was designed through extensive *in vitro* and *in vivo* testing (chapter 3 and 4). In contrast to other polymers, PDLLCL was not associated with release of damage-associated molecular patterns by islets and supported islet functionality. Furthermore, *in vivo*, PDLLCL elicited a minor tissue response that was dampened within 28 days after subcutaneous implantation. After this period the polymer was also adequately vascularized and supported islet transplantation (chapter 4).

Here we studied the quality of the metabolic control of an allogenic rat islet graft transplanted into a subcutaneous placed PDLLCL scaffold in a diabetic rat model. To this end, we used nude rats with streptozotocin-induced diabetes and compared the metabolic function with islet grafts under the kidney capsule.

Materials and methods

Experimental design

Streptozotocin-induced diabetic Rowett nude rats received a graft of islets isolated from Albino Oxford (AO) rats. Grafts were either transplanted under the kidney capsule or in a subcutaneously pre-implanted PDLLCL (poly($^{68}/_{32}$[$^{15}/_{85}$ $^{D}/_{L}$-lactide]-co-ε-caprolacton) scaffold. The kidney capsule grafts served as controls. Grafts were composed of 10 μl of endocrine tissue, which is the equivalent of the endocrine rat pancreas [10]. To monitor graft function, nonfasting blood glucose levels were measured weekly and between 8 and 10 weeks after transplantation an oral glucose tolerance test (OGTT) was performed. Islet grafts of both groups were retrieved four months after islet transplantation to study islet survival. Histology was applied to confirm the absence of beta-cells in the native pancreas, defined as <5% of normal controls.

Preparation PDLLCL scaffold

Porous PDLLCL (Polyganics B.V. (Groningen, The Netherlands) scaffolds were prepared by salt leaching. Briefly, PDLLCL was dissolved in chloroform (Sigma-Aldrich, Zwijdrecht, The Netherlands) at a concentration of 4% (w/v). Natriumchloride (NaCl) particles (250 – 425 μm; Sigma-Aldrich) were added (10:1 w/w) to the PDLLCL solution while homogenously mixed. The PDLLCL/NaCl solution was transferred into sterile glass petri dishes and left for 24 hours in the fume hood to allow the chloroform to evaporate. The NaCl particles were removed from the 5 mm thick PDLLCL sheet by extensively washing with sterile water. The porous PDLLCL sheet was resized into approximately 10 mm by 15 mm rectangle-shaped scaffolds. During the casting process two channels were created by introducing 400 μm iron rods. After casting these iron rods were substituted by hydrophobic polyethylene tubing (0.5 x 1 mm; Brinkman, 's-Gravenzande, The Netherlands) that keep the channels patent during the four weeks engraftment of the scaffold before islets were transplanted. The scaffolds were washed and stored in 70% ethanol

for several days to ensure sterility.

To facilitate ingrowth of blood vessels and mimic the pancreatic environment, the PDLLCL scaffold was embedded in rat fibrin before implantation. Rat fibrinogen (2 mg/ml; Stago, Leiden, The Netherlands) was dissolved in CMRL (Gibco; Life Technologies, Bleiswijk, The Netherlands) containing 50 U/ml Penicillin/Streptomycin (Gibco). To solidify the fibrinogen in the pores of the scaffold, 1.0 U/ml thrombin IIa (Stago) was added and the fibrin gel was incubated subsequently 1 hour at room temperature and 1 hour at 37 °C.

Animals

The University of Groningen Institutional Animal Care and Use Committee approved all described animal procedures. Male AO rats weighing approximately 250 – 350 gram served as islet donors. This rat strain was obtained from our own breeding colony. Male Rowett nude rats (Hsd:RH-Foxn1$^{rnu/rnu}$) with an age of 7-10 weeks obtained from Harlan (Horst, The Netherlands) were used as transplant recipients. Animals were fed standard chow and had ad libitum access to water.

Islet isolation

Islets were harvested from pancreata of AO rats according to the Serva protocol (Heidelberg, Germany) [11]. Briefly, after cannulation of the bile duct [12], the pancreas was infused with a NB8 collagenase solution (Serva) dissolved in Hank's balanced salt solution (HBSS; Gibco) containing DNAse (Sigma-Aldrich). The pancreas was carefully excised and transferred to a sterile flow cabinet. The digestion was completed with an incubation of 18 minutes at 37 °C of the pancreas in the collagenase/DNAse solution. Islets were purified from the exocrine tissue by a histopaque (Sigma-Aldrich) density gradient. Islets were handpicked and counted. The total islet volume was determined by measuring the diameter of islets in a 2% aliquot of the total islet yield [13].

Islet transplantation

Rowett nude rats received a streptozotocin IV (Sigma-Aldrich) injection of 60 mg/kg to become diabetic. Diabetes was confirmed by two consecutive measurements of blood glucose above 20 mmol/L during a period of 14 days using an Ascensia contour blood glucose meter (Bayer, Mijdrecht, The Netherlands). After diabetes confirmation, a Linplant insulin tablet (LinShin, Scarborough, Canada) was subcutaneously implanted. One month before islet transplantation, scaffolds were subcutaneously implanted on the back of the diabetic rats (n = 4) to allow vascularization of the scaffold and dampening of the foreign-body response. Immediately after islet isolation, a small incision was made at the side of the scaffold. The PE tubing was removed from the scaffold, creating two channels for islets. An islet graft of 10 µl was divided over the two channels with a 23G Hamilton syringe (VWR International B.V., Amsterdam, The Netherlands). The insulin tablet was removed directly after islet transplantation. The rats received a single injection of Buprenorphine (0.01 mg/kg; Sigma-Aldrich) as pain medication.

To compare the scaffold with a well-established islet transplantation site in rodents, islets were transplanted under the kidney capsule of diabetic Rowett rats (n = 6) as described before [14]. Briefly, a small incision was made in the skin and the peritoneum, just above the kidney, to access the kidney capsule. With a needle an opening in the kidney capsule was created, subsequently a metal rod was used to obtain a subcapsular pocket under the upper pole for islet transplantation. Control rats were transplanted with an islet graft of 10 µl and also received a single injection of pain medication.

Post-transplantation monitoring

During the 4 months after transplantation, nonfasting blood glucose levels and weight of the rats were measured every week. Animals were considered to be normoglycemic when nonfasting blood glucose levels were below 10 mmol/L. When on 2 consecutive days a blood

glucose level was measured of 20 mmol/L or higher, the animal was euthanized by a blunt incision through the heart and the scaffold or kidney was removed for histological analysis.

Oral glucose tolerance test (OGTT)

Graft function and innervation was tested by an OGTT within 8 to 10 weeks after transplantation. For this test, the rats were fasted for 5 hours and habituated to consume 2 gram of rat chow (RM1; SDS diets, Essex, United Kingdom) within 5 minutes. Blood samples were taken from the tail vein just before the start of the test (-5) and 1, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120 minutes after consuming the meal. At the same time points, blood glucose was measured with the Ascensia contour blood glucose meter. Blood was collected in EDTA dipotassium salt tubes (Microvette CB 300 K2E; Sarstedt, Nümbrecht, Germany). After centrifugation, plasma was used to measure insulin concentrations with an ultrasensitive rat insulin ELISA kit (Crystal Chem, Inc., Downers Grove, USA) according to manufactures instruction. The optical density was measured at 450 nm within 30 minutes after adding the stop solution using a microplate reader (VersaMax; Molecular Devices, Berkshire, United Kingdom). The area under the curve (AUC) was used as measure for glucose tolerance [15]. In addition, the insulin:glucose ratio was determined at the time point of the highest insulin peak.

Histology

Four months after transplantation, the implanted scaffold was harvested with surrounding tissue and from the control group the kidney with the graft was excised. The scaffolds were fixated in 2% paraformaldehyde and embedded in glycol methacrylate (GMA; Technovit® 8100; Heraeus Kulzer GmbH, Wehrheim, Germany). The kidneys were fixated in formalin and processed for paraffin embedding. To exclude endocrine pancreas regeneration, pancreas biopsies were taken of animals in both groups. These biopsies were fixated in Bouin's solution (Sigma-Aldrich) and embedded in paraffin.

The GMA embedded scaffolds were sectioned at 2 µm and processed for insulin staining. Briefly, sections were dried at 37°C and incubated in 0.01% trypsin (in 6.8 mM 0.1% $CaCl_2$ and 0.1 M Tris-HCl, pH 7.8) for 10 min at 37°C. The sections were incubated with a mouse anti-rat insulin antibody (Sigma-Aldrich; 1:300 in PBS + 1% BSA) for 2 hours at 37°C. After this incubation, the staining procedure was performed at 20°C. Nonspecific binding was blocked by a 5-minute incubation with 10% normal goat serum. The rabbit anti-mouse alkaline phosphatase conjugated secondary antibody (Dako, Heverlee, Belgium; 1:100 in PBS + 1% BSA) was applied for 45 minutes. Alkaline phosphatase activity was demonstrated by incubating the sections for 10 minutes with SIGMAFAST™ Fast Red (Sigma-Aldrich). A short incubation with hematoxylin was used as counterstain.

Kidney sections were stained as well for insulin. Briefly, sections were incubated with a mouse anti-rat insulin antibody (1:300 in PBS + 1% BSA) for 1 hour at room temperature. Nonspecific binding was blocked by 15 minutes incubation with 10% normal rabbit serum. The secondary rabbit anti-mouse alkaline phosphatase conjugated antibody (1:100 in PBS + 1% BSA) was applied for 30 minutes. Alkaline phosphatase activity was demonstrated by incubating the sections for 10 minutes with SIGMAFAST™ Fast Red. A short incubation with hematoxylin was used as counterstain.

The paraffin pancreas biopsies were sectioned at 5 µm and stained with aldehyde fuchsin to determine the presence of viable of beta cells [16]. Briefly, sections were oxidized by 2.5% $KMnO_4$ (Sigma-Aldrich) and 5% H_2SO_4 (Merck Millipore, Amsterdam, The Netherlands) in demineralized water for 90 seconds and cleaned with 2% oxalic acid (Sigma-Aldrich) for 2 minutes. After 2 minutes incubation with 70% ethanol, sections were incubated with aldehyde fuchsin solution [16,17] for 10 minutes. Sections were counterstained with Halmi during 6 minutes incubation. Sections were analyzed using a Leica DM 2000 LED microscope with a Leica DFC 450 camera (Leica Microsystems B.V.).

Statistics

Statistical analysis was carried out in GraphPad Prism (version 5.0b; GraphPad Software, Inc., La Jolla, USA). A Shapiro-Wilk normality test was performed to test the data for normality. In

the case of parametric distribution, an unpaired t test was performed and data were presented in mean ± standard deviation. In case of nonparametric distribution, a Mann-Whitney test was performed and data was presented in median ± interquartile range. P-values < 0.05 were considered significant.

Table 1: Islet characteristics before transplantation.

The mean ± standard error of mean of the endocrine volume (µl) and islet number before transplantation under the kidney capsule or in the scaffold.

	Kidney capsule	Scaffold
Endocrine volume (µl)	11.3 ± 1.5	9.9 ± 1.6
Islets (number)	3313 ± 253	3456 ± 236

Results

Immediate restoration of normoglycemia

Islets grafts with an endocrine volume of 11.3 ± 1.5 µl or of 9.9 ± 1.6 µl were respectively transplanted under the kidney capsule and in the scaffold (Table 1). Complete normoglycemia, defined as nonfasting blood glucose levels below 10 mmol/L, was restored within 1 week in both the kidney capsule and the scaffold group (Figure 1). Islet transplantation under the kidney capsule resulted in a fairly stable blood glucose level of 3.5 ± 0.1 mmol/L (mean ± standard error of the mean) throughout the study period. The blood glucose level of the scaffold-transplanted animals was statistically significantly ($p < 0.05$) higher (4.6 ± 0.2 mmol/L). However, the blood glucose levels of the scaffold-transplanted animals fluctuated more during the whole study period. Animals of both groups remained normoglycemic up to 16 weeks after islet transplantation. Furthermore, animals of both groups gained weight and

their overall health improved after transplantation (Figure 2). Scaffold-transplanted animals gained statistically significantly ($p < 0.05$) more weight then animals of the kidney capsule group.

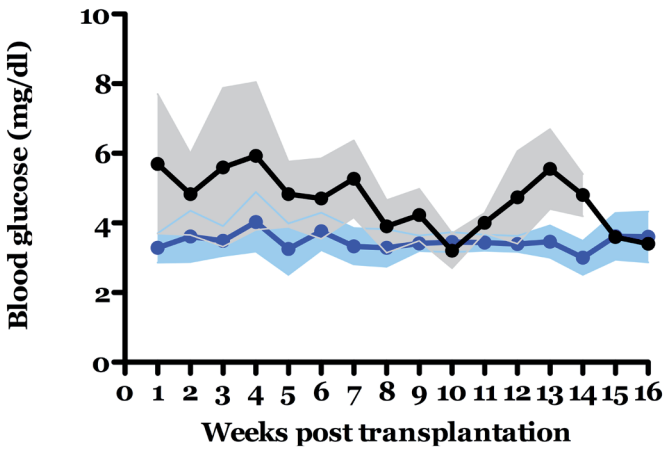

Figure 1: Long-term blood glucose levels after islet transplantation under the kidney capsule and in the scaffold.

Nonfasting blood glucose measurements after islet transplantation under the kidney capsule (blue line) or in a prevascularized subcutaneous scaffold (black line). Data points represent blood glucose mean ± standard error of the mean (n=6 kidney capsule, n=4 scaffold).

No significant differences in OGTT of kidney capsule and scaffold groups

To test the metabolic function of the islet grafts an OGTT was performed between 8 and 10 weeks after transplantation (Figure 3). Islet grafts in the scaffold under the skin induced a different regulation than islets under the kidney capsule. Islet grafts under the kidney capsule responded faster with an insulin release upon consumption of the meal (Figure 3A). Directly after consumption of the meal we observed a rise in insulin, which occurred only 10 minutes after meal consumption in the recipients of grafts in the subcutaneous scaffold. The concomitant increase in glucose levels also started later and reached in recipients of a graft

under the skin 8.7 ± 0.2 mmol/L glucose after 30 minutes (Figure 3B). The glucose peak of the kidney capsule group was after 10 minutes (5.6 ± 0.5 mmol/L) and was lower than in the scaffold. The insulin:glucose ratio of the kidney capsule group at this peak was 0.5 (0.4 – 0.7), whereas this ratio was two times reduced in the scaffold group (0.2 (0.2 – 0.3)) at their peak. The glucose clearance of the kidney capsule group (Figure 3C) was 248 (mmol/L)*minutes (185 – 278) (median ± interquartile range). A two-fold increase of glucose clearance was measured when transplanting islets into the subcutaneous scaffold (522 (mmol/L)*minutes (250 – 632)). This increase was not observed in the insulin values after an oral glucose load (Figure 3D). During the OGTT, the kidney capsule group released 215 (ng/ml)*minutes (198 – 245) insulin and this was 193 (ng/ml)*minutes (175 – 195) for the scaffold group.

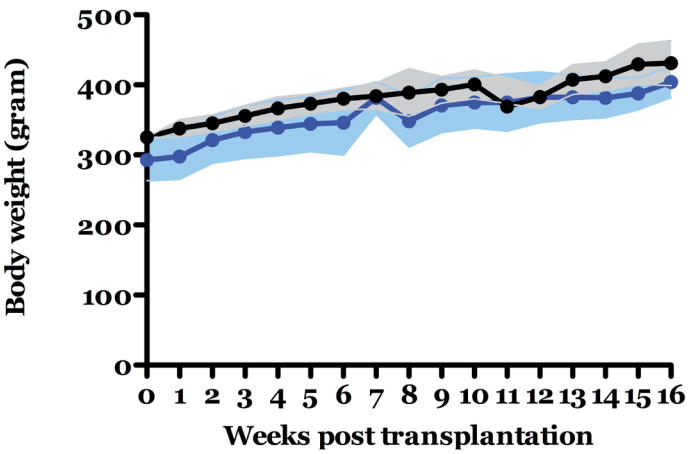

Figure 2: Weight of the rats after islet transplantation under kidney capsule or in scaffold

The weight (grams) of rats from the kidney capsule (blue line) and scaffold (black line) groups was measured on a weekly basis after islet transplantation for the duration of the study. Data points represent blood glucose mean ± standard error of the mean (n=6 kidney capsule, n=4 scaffold).

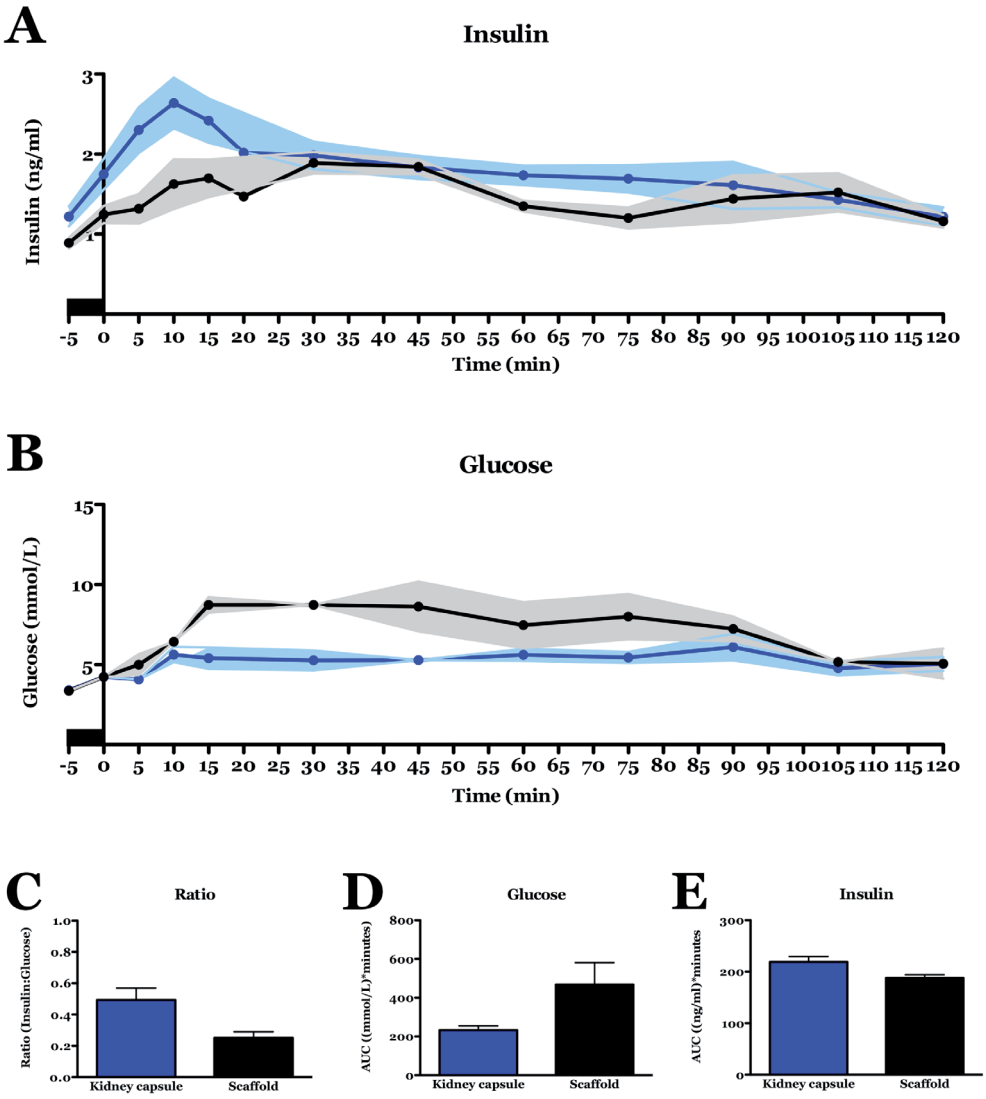

Figure 3: The metabolic capacity of islet transplanted under kidney capsule or in scaffold.

The insulin (A) and glucose (B) levels of the kidney (blue line) and scaffold (black line) groups after a meal (black rectangle). The insulin and glucose levels are plotted as mean ± standard error of mean. The Insulin:Glucose ratio was determined at the time point of the highest insulin peak (C). The glucose clearance from the blood expressed as area under the curve ((mmol/L)*minutes) for the kidney capsule

and the scaffold (D). The release of insulin in the blood during the oral glucose tolerance test expressed as area under the curve ((ng/ml)*minutes) for the kidney capsule and the scaffold (E). This metabolic capacity was measured between 8 and 10 weeks after islet transplantation. For figure 3C,D, and E the median and interquartile range are plotted (n=6 kidney capsule, n=3 scaffold).

Insulin positive islets under kidney capsule and in scaffold 16 weeks post transplantation

After 16 weeks, the islet containing kidneys and scaffolds were carefully excised and processed for histology. Large numbers of insulin positive islets were found in both the kidney capsule and scaffold groups (Figure 4). Islets transplanted in our preimplanted scaffold had normal spherical shapes and were not organized in sheet shape as observed under the kidney capsules. Islets were found in different sizes and were lined in the channels of the porous scaffold. The absence of beta-cells in the native pancreas, defined as <5% of normal controls, was always histologically confirmed at the time of death.

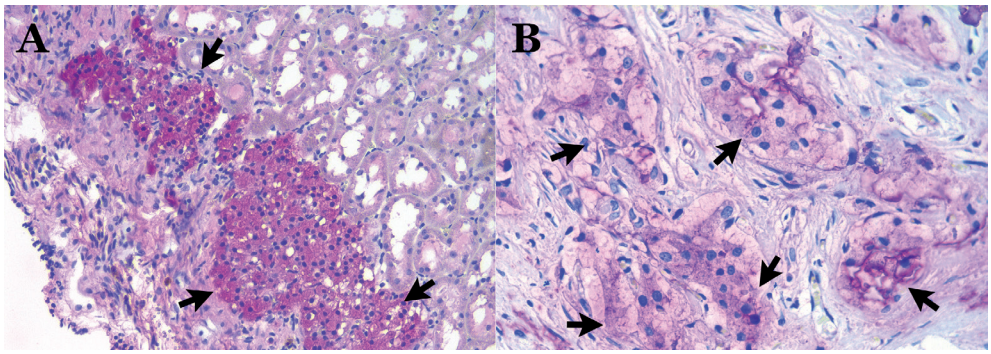

Figure 4: Histological analysis of islets transplanted under the kidney capsule or in the PDLLCL scaffold.

Insulin positive islets (SIGMAFAST™ Fast Red, arrows) were found under the kidney capsule (A, 20x) and in the scaffold (B, 40x) 16 weeks after transplantation.

Discussion

This study indicates that our PDLLCL scaffold is suitable for generating a prevascularized islet transplantation site that supports metabolic control in a diabetic rat model. Allogenic rat islet transplantation into the PDLLCL scaffold after four weeks of prevascularization under the skin resulted in the restoration of nonfasting blood glucose homeostasis for up to four months. Furthermore, the islets conveniently survived in the scaffold as large amounts of insulin positive cells were found with histological analysis at the end of the study. However, islet transplantation under the kidney capsule seems to be slightly more efficient than islet transplantation into the subcutaneous scaffold. Nonfasting blood glucose levels were more tightly regulated in the kidney capsule group, more fluctuations were observed in the scaffold group. The OGTT also showed a better metabolic control after transplantation under the kidney capsule, but no differences were found in islet survival between both groups.

The difference in metabolic control between grafts in the scaffolds and under the kidney capsule was evident in the insulin:glucose ratio. The insulin:glucose ratio indicates that glucose was cleared more efficient in recipients of an islet grafts under the kidney capsule as glucose was metabolized with a prompt increase of plasma insulin levels compared to recipients under de skin. Grafts under the skin responded slower with releasing insulin, resulting in a higher glucose peak than the kidney capsule recipients. Up to now only a few studies have focused on glucose clearance in relation to transplantation site [18-20]. However, our observations are consistent with previous rodent studies that compare islet transplantation under the kidney capsule with islets in a subcutaneous transplantation site [18,19]. These studies also observe differences in glucose metabolism between the two transplantation sites. Although, vascularization of the subcutaneous site is established before transplantation in all studies, the difference in glucose metabolism is attributed to the differences in blood supply to the kidneys and the skin. The kidneys receive 20% of the total cardiac output, whereas the

skin receives 7.5% [21]. This implies a slower and lower supply of blood with elevated glucose to the skin grafts with slower appearance of insulin in the circulation as a consequence.

A remarkable finding was the observation that animals with an islet graft transplanted under the skin gained more weight than the recipients of an islet graft transplanted under the kidney capsule. Insulin-like growth factors (IGFs), such as IGF-1 and IGF-2, play a key role in lipid metabolism and body weight regulation in both humans and animals [22,23]. These two growth factors are under control of hormones such as insulin [23]. The OGTT showed that the insulin secretion is lower in the scaffold group compared with the kidney capsule group, suggesting that there is less insulin to regulate the IGFs. Lower insulin levels are associated with lower IGFs [22,23]. As a result of low IGFs levels, the lipid metabolism and body weight regulation is disturbed. It has been shown that low levels of IGF-2 in humans result in weight gain [22]. Therefore, this can explain why the scaffold animals gained more weight than the kidney capsule animals. In follow up studies we will study the IGF levels in the recipients.

PDLLCL is a Food and Drug Administration (FDA) approved polymer, which has shown to induce only minimal tissue responses in animals and humans when applied for wound repair, recovery of nerve defects, or as anti-adhesive sheet [24-26]. We demonstrated the ability to use PDLLCL as scaffold material to control the glucose metabolism after subcutaneous islet transplantation in a diabetic rat model. Determination of the marginal islet dose for our subcutaneous, prevascularized, replaceable scaffold in other animal models is warranted for future clinical application.

References

1. Tong LM, Fong H, Huang Y. Stem cell therapy for Alzheimer's disease and related disorders: current status and future perspectives. Exp Mol Med. 2015;47: e151.

2. Gonzalez C, Bonilla S, Flores AI, Cano E, Liste I. An update on human stem cell-based therapy in Parkinson`s disease. Curr Stem Cell Res Ther. 2015.

3. Janowski M, Wagner DC, Boltze J. Stem Cell-Based Tissue Replacement After Stroke: Factual Necessity or Notorious Fiction? Stroke. 2015;46: 2354-2363.

4. Hsieh MM, Fitzhugh CD, Weitzel RP, Link ME, Coles WA, Zhao X, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. JAMA. 2014;312: 48-56.

5. Lebherz-Eichinger D, Schwarzer R, Motal MC, Klaus DA, Mangold A, Ankersmit HJ, et al. Liver transplantation reverses hypergammaglobulinemia in patients with chronic hepatic failure. Biochem Med (Zagreb). 2015;25: 252-261.

6. Hering BJ. Achieving and maintaining insulin independence in human islet transplant recipients. Transplantation. 2005;79: 1296-1297.

7. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005;293: 830-835.

8. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343: 230-238.

9. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005;5: 2037-2046.

10. van Suylichem PT. Rat islet isograft function. Effect of graft volume and transplantation site. Transplantation. 1994;57: 1010-1017.

11. Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, Brendel MD, et al. Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. Cell Transplant. 2013;22: 1463-1476.

12. de Haan BJ, Faas MM, Spijker H, van Willigen JW, de Haan A, de Vos P. Factors influencing isolation of functional pancreatic rat islets. Pancreas. 2004;29: e15-22.

13. de Vos P, Hillebrands JL, de Haan BJ, Strubbe JH, van Schilfgaarde R. Efficacy of a prevascularized expanded polytetrafluoroethylene solid support system as a transplantation site for pancreatic islets.

Transplantation. 1997;63: 824-830.
14. Visser L, Poppema S, de Haan B, Klok P, van der Leij J, van den Berg A, et al. Prolonged survival of rat islet xenografts in mice after CD45RB monotherapy. Transplantation. 2004;77: 386-391.
15. Martin D, Bellanne-Chantelot C, Deschamps I, Froguel P, Robert JJ, Velho G. Long-term follow-up of oral glucose tolerance test-derived glucose tolerance and insulin secretion and insulin sensitivity indexes in subjects with glucokinase mutations (MODY2). Diabetes Care. 2008;31: 1321-1323.
16. Smelt MJ, de Haan BJ, Faas MM, Melgert BN, de Haan A, de Vos P. Effects of acute cytomegalovirus infection on rat islet allograft survival. Cell Transplant. 2011;20: 1271-1283.
17. de Vos P, van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, de Haan BJ, et al. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes. 1999;48: 1381-1388.
18. Tatarkiewicz K, Hollister-Lock J, Quickel RR, Colton CK, Bonner-Weir S, Weir GC. Reversal of hyperglycemia in mice after subcutaneous transplantation of macroencapsulated islets. Transplantation. 1999;67: 665-671.
19. Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AM. A prevascularized subcutaneous device-less site for islet and cellular transplantation. Nat Biotechnol. 2015;33: 518-523.
20. Cornolti R, Figliuzzi M, Remuzzi A. Effect of micro- and macroencapsulation on oxygen consumption by pancreatic islets. Cell Transplant. 2009;18: 195-201.
21. Smith JJ, Kampine JP. Circulatory physiology: the essentials. 3rd ed: Williams & Wilkins; 1990.
22. Heald AH, Karvestedt L, Anderson SG, McLaughlin J, Knowles A, Wong L, et al. Low insulin-like growth factor-II levels predict weight gain in normal weight subjects with type 2 diabetes. Am J Med. 2006;119: 167.e9-167.15.
23. Gomes RJ, Leme JA, de Moura LP, de Araujo MB, Rogatto GP, de Moura RF, et al. Growth factors and glucose homeostasis in diabetic rats: effects of exercise training. Cell Biochem Funct. 2009;27: 199-204.
24. Meek MF, Coert JH. US Food and Drug Administration /Conformit Europe- approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. Ann Plast Surg. 2008;60: 466-472.
25. Meek MF, Den Dunnen WF. Porosity of the wall of a Neurolac nerve conduit hampers nerve regeneration. Microsurgery. 2009;29: 473-478.
26. Bertleff MJ, Meek MF, Nicolai JP. A prospective clinical evaluation of biodegradable neurolac nerve guides for sensory nerve repair in the hand. J Hand Surg Am. 2005;30: 513-518.